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(54) Title: STABILIZING cGMP IN AQUEOUS FORMULATION

(57) Abstract: The present invention relates to a method of stabilizing caseino-glycomacropeptide (cGMP) in aqueous formulations and reducing an off-flavor formation. In particular, the present invention comprises a formulation, having a pH below about 6 and/or comprising a hydrophobic resin and/or an off-flavor masking substance and/or agents blocking functional groups in the caseino-glycomacropeptide.

Stabilizing cGMP in aqueous formulations

The present invention relates to a method of stabilizing caseino-glycomacropeptide (cGMP) in aqueous formulations and reducing an off-flavor formation. In particular, the present invention comprises a formulation, having a pH below about 6 and/or, comprising a hydrophobic resin and/or an agent blocking functional groups in the caseino-glycomacropeptide.

Caseino-glycomacropeptide (cGMP) is a glycosylated compound formed during the enzymatic cleavage of kappa-casein from the milk of mammals by the action of rennet or pepsin. To obtain this compound as a starting material, e.g. an acidic casein or a caseinate hydrolyzed by rennet, or even a demineralized, lactose-free sweet whey, is treated with trichloroacetic acid to precipitate the proteins, the supernatant is collected and dialyzed, and finally, the dialysate is dried.

So as to obtain cGMP on an industrial scale acidic casein or sodium or calcium caseinate is treated with rennet which results in the coagulation of para-kappa-casein. The supernatant is then acidified to a pH of about 4 - 5 in order to precipitate the calcium phospho-caseinate. After separation of the precipitate, the solution is neutralized, demineralized by reverse osmosis, and finally concentrated and dried. Other processes include flocculating whey proteins from whey emanating from cheese production, recovering the supernatant and ultrafiltrating the supernatant using membranes having a cutoff threshold of approximately 15,000 Dalton, thus producing a retentate containing the sialo-glycoproteins.

The cGMP thus obtained is utilized in a variety of different applications, such as in a supplement to nutritional formulas as anti-thrombotic, anti-diarrhoeal compound and for special amino acid diets. Due to its microbizidal activity cGMP is also utilized in formulations for treating bacteria in the buccal cavity which are responsible for the formation of dental plaque and caries. It has been found that the capacity of Actinomyces strains and

Streptococcus strains, bacteria populating the buccal cavity and considered to be involved in the initiation and formation of dental plaque, to adhere to buccal epithelial cells, to the surface of teeth coated with saliva and to form co-aggregates with one another may be reduced by providing cGMP in dental formulations, thus diminishing the detrimental effects of said bacterial strains. In addition, cGMP is also described to participate in the effect of a remineralization of demineralized portions of tooth structures.

One of the disadvantages of such formulations, however, resides in that an off-flavor develops during storage thereof. To solve this problem the art has proposed to include binding proteins in the formulations, such as antibodies, as a means of controlling the perceptibility of odoriferous materials which may be present, more specifically undesirable flavors or fragrances or constituents thereof.

Yet, proceeding accordingly is still cumbersome and due to the materials involved also expensive.

An object of the present invention therefore resides in overcoming the shortcomings of the prior art and to provide a cGMP containing formulation, that exhibits an extended shelf life without developing off-flavor.

During the extensive studies leading to the present invention, the inventors achieved to solve this problem by providing a cGMP containing aqueous composition comprising, a hydrophobic resin and an agent, that blocks specific functional groups in cGMP, responsible for off-flavour formation, and/or by adjusting the pH of the composition to a value of less than about 7.

Surprisingly, an extension of the shelf-life of cGMP containing products may already be obtained by simply lowering the pH-value of the product below about 6. However, for many products, in particular for compositions which are intended to be used on the skin or in the orifice a higher pH-value is desirable. In order to be able provide also cGMP-containing products with a pH-value above about 6, having an extended shelf-life, the present invention

proposes the addition of a hydrophobic resin and of an agent blocking the functional groups in cGMP.

One of the main advantages of the present invention is that both, the addition of a hydrophobic resin and of an agent blocking the functional groups in cGMP and the lowering of the pH-value are complementing each other. Depending on the chosen final product and/or the desired shelf-life, a person skilled in the art may obtain stable products by tuning both, the pH-value and the amount of hydrophobic resin and blocking agent to be added. Thus, the present invention offers not only the possibility to stabilize the composition, but also to minimize the amount of the respective additives, by decreasing the pH of the product correspondingly. The minimization of food additives is very desirable both economically and in view of the acceptance of the product, as products having an low amount of food additives are highly estimated by the customers.

Additionally, the composition of the present invention not only exhibits an extended shelf-life, but surprisingly also provides an increased stability and an essentially reduced off-flavor formation, when exposed transiently during storage or transportation to temperatures above room-temperature.

In a second aspect the present invention provides a method of producing a composition, which comprises preparing a composition comprising cGMP, adding an agent, that chemically blocks functional groups in cGMP and a hydrophobic resin, and/or adjusting the pH-value to a value in the range of from about 3 to about 6.

In a third aspect the present invention provides use of the composition in the manufacture of a medicament or a composition for treating or preventing caries, plaque formation, dental diseases, diseases of the mouth cavity or gums.

Preferably, said hydrophobic resin may be selected from the group consisting of Serdolith III, Lewatit EP-63, Lewatit OC 1064, Lewatit OC 1066, Lewatit VC-OC or Amberlite XAD. In a preferred way, a food-tolerable substance is used instead of hydrophobic resin, such as

chlorophillin, sodium octenyl succinate starch, hydroxypropyl methyl cellulose or casein. Without being bound to any theory it may be supposed that said hydrophobic resin is acting as a sorbens trapping certain off-flavor substances. The amount of the hydrophobic resin may be selected in the range of from 0.01 to about 5 wt.-%, preferably from 0.05 to about 5 wt.-%, more preferably from 0.1 to about 2 wt.-%, each based on the final product.

The blocking or masking agent may be chosen from any acid anhydride, that may be included in an aqueous formulation, or derivatives thereof. Preferred examples may be selected from the group consisting of succinic anhydride, maleic anhydride, propio-lactone, chlorophillin and derivatives thereof, such as there isomeric forms. In the context of this application the term "derivatives thereof" comprises any compound derived from the above mentioned components by e.g. substituting moieties, as long as the activated acid component, i.e. the anhydride element remains. When utilized in a food product the blocking agent is preferably a food-grade chemical compound. Without being bound to any theory, it is supposed that said acid anhydrides react with chemical moieties of cGMP, in particular with amino groups, and thus prevent e.g. Maillard reactions or Strecker degradation reactions. The amount of the blocking agent is in the range of from about 0.005 to 1 wt.-%, preferably 0.01 to 1 wt.-%, more preferably 0.01 to 0.6 wt.-%, more preferably 0.1 to 0.5 wt.-%, each based on the final product.

The pH of the final product or composition is in the range of from about 3 to about 7, preferably in the range of from about 4 to about 6. For an lowering of the pH-value organic or inorganic acids or acidic buffer systems may be used, in particular e.g. aqueous HCl, H_3PO_4 and acetic acid.

The composition contemplated by the present invention may be any aqueous formulation, preferably any composition having a water activity between 0.2 - 1, since in these formulations the detrimental effects of a degradation and/or off-flavor development are prominent. The invention is particularly suited for compositions having a water activity of between 0.7-0.9, more preferably of about 0.8. The term "water activity" is to be understood as defined in e.g. Food Chemistry, Belitz H.D., Grosch W., (1999) p.4-6, Springer. The

measurement of water activity was performed on a Hygroskop DT (Rotronic AG, Zürich, Switzerland).

In principle, the composition of the present invention may be any food or pharmaceutical product containing cGMP, in particular a food product having a sweet taste due to the presence of sugars or sugar substitutes, which tend to be involved in Maillard reactions (that result in an off-flavor of the product), dairy products, such as e.g. an infant formula or a pharmaceutical product, in particular a pharmaceutical product for treating or preventing dental problems, such as e.g. caries or plaque formation, or a cosmetic or an oral composition.

According to a preferred embodiment, the composition of the invention may be a product for oral hygiene or a product for any application in the mouth cavity and/or throat, in particular a tooth paste, a gel, a tooth powder, a mouth wash, a chewing gum, a tablet or a lozenge. In particular, the composition may also be a product for oral hygiene which is present in pre-applied form on any dental cleaning means, such as dental floss.

A preferred embodiment of the invention is a composition comprising cGMP, Serdolith III, and succinic anhydride or maleic anhydride.

The following examples illustrate the invention in a more detailed manner. It has, however, to be understood that the present invention is not limited to the examples but is rather embraced by the scope of the appended claims.

Example 1

Preparation of a cGMP basis composition

A cGMP basis composition consisting of 39 wt.-% glycerol, 10 wt.-% cGMP, 0.002 wt.-% chlorohexidine (in this model added as preservative against microbial growth) and water was prepared. The resulting basis composition has an water activity value a_w of 0.8, which was determined according to manufacturer instructions (Hygroskop DT, Rotronic AG, Zürich,

Switzerland).

Example 2

pH-dependent off-flavor formation

Samples of the cGMP basis composition according to Example 1 were taken and the pH value of each of said samples was adjusted by adding either 1 M hydrochloric acid or 2 M sodium hydroxide to a pH-value in the range of between 5.5 and 8.0. All samples were stored at 49°C for 3 weeks and were subjected subsequently to organoleptic tests.

No off-flavor was organoleptically detectable in samples having a pH-value of less than 6. During said organoleptic tests, test persons evaluated the odor of the samples adjusted to different pH-values.

These results were confirmed by a volatile flavor analysis by GC-MS. The volatile flavor compounds can be extracted according to the method described by De Frutos M, Sanz J, Martinez-Castro I, (1988) Chromatographia, 25, 861-864. The GC-MS separation and identification was performed accordingly: GC - Hewlett Packard 5890 II, MS – Hewlett Packard 5972, capillary column – Supelcowax 10, 60m x 0.25 mm, 0.15 µm film thickness, Flow – 1ml helium /min, Injection volume – 1µl cold on-column, Temperature gradient – 35°C, 50°C/min to 60°C, 4°C/min to 150°C, hold for 4 min, 10°C/min to 240°C and hold for 20min, the NIST MS spectra library was used for substance identification. No peaks indicative for a known cGMP degradation product or off-flavor substance in substantial amounts could be detected in samples having a pH-value of less than about 6.

Additionally, also a comparison of the HPLC finger print of a freshly prepared cGMP basis composition and of the above-described samples was performed. Essentially, no changes were observed in the HPLC finger print of samples having a pH-value of less than about 6. The analytical conditions for the separation of cGMP by HPLC were the following: HPLC – Agilent 1100, Quaternary pump, diode array detector at 215nm, injection volume – 25µl, column – TSK Gel Super ODS, 2µm, 110A, 2 x 4.6mm and 100 x 4.6mm, column

temperature 50°C, mobile phase – A) 0.05% trifluoric acid in water, B) 0.035% trifluoric acid in acetonitrile, flow 2.5 ml/min, solvent gradient – 20% B to 40% B in 6min, 40% B to 50% B in 1.5min, 50% B to 95% B in 0.5min, hold for 0.5min, 95% B to 20% B in 1.5min and hold for 2min.

Example 3

Effects of the addition of blocking agent or hydrophobic resin on the off-flavor formation

0.4 wt.-% of succinic anhydride anhydride (Merck GmbH, Darmstadt, Germany) or 2 wt.-% of Serdolith III (Fluka, Buchs, Switzerland) were added to the cGMP basis composition obtained according to Example 1. Samples were taken, the pH of said samples was adjusted as described in Example 2 to a value of 6.8 respective 6.5 and said samples were stored as described in Example 2.

No off-flavor formation could be detected organoleptically (experimental proceeding, see Example 2) or via GC-MS (experimental proceeding, see Example 2) even in samples having a pH-value of of 6.8 respective 6.5, thus having a pH-value of above 6. For control, otherwise identical samples without the above-mentioned blocking agent and hydrophobic resin were prepared which had a detectable off-flavor in case of an pH-value of above 6.

Example 4

Effects of the pH-adjustment or the addition of blocking/masking agent or hydrophobic resin on the off-flavor formation of dental care products containing cGMP

Samples of commercially identical dental care products were taken, cGMP was added and the pH value of each of said samples was adjusted by adding either 1 M hydrochloric acid or 2 M sodium hydroxide to a pH-value in the range of between 5.0 and 7.5. All samples were stored at 49°C for 3 weeks and were subjected subsequently to organoleptic tests.

No off-flavor was organoleptically detectable in samples having a pH-value of less than 6. During said organoleptic tests, test persons evaluated the odor of the samples adjusted to

different pH-values.

In a similar experiment, 0.25 wt.-% of succinic anhydride (Merck GmbH, Darmstadt, Germany) or 0.25 wt.-% of maleic acid anhydride (Fluka, Buchs, Switzerland) or 0.1 wt.-% propio-lactone (Acros, Chemie Brunschwig, Basel, Switzerland) or 0.01 wt.-% chlorophillin or 1 wt.-% of Levatit OC 1066 (Fluka, Buchs, Switzerland) were added to the cGMP containing dental care product composition. The pH of said samples were adjusted as described in Example 2 to a value of 7.0 and said samples were stored as described in Example 2.

No off-flavor formation could be detected organoleptically (experimental proceeding, see Example 2) even in samples having a pH-value of 7.0, thus having a pH-value of above 6. For control, otherwise identical samples without the above-mentioned blocking agent and hydrophobic resin were prepared which had a detectable off-flavor in case of a pH-value of above 6.

Claims

1. A cGMP containing aqueous composition exhibiting a reduced off-flavor even after long storage, comprising

   (i) a hydrophobic resin; and/or

   (ii) an agent, that chemically blocks functional groups in cGMP;

   and/or

   (iii) the pH of the composition is below about 7.

2. The composition according to claim 1, wherein the hydrophobic resin is selected from the group consisting of Serdolith III, Lewatit EP-63, Lewatit OC 1064, Lewatit OC 1066, Lewatit VC-OC or Amberlite XAD.

3. The composition according to claim 1 or 2, wherein the blocking or masking agent is selected from the group consisting of succinic anhydride, maleic anhydride, propiolactone, chlorophillin or derivatives thereof.

4. The composition according to any of the preceding claims, wherein the pH of the final product is in the range of from about 3 to about 7.

5. The composition according to any of the preceding claims, wherein the amount of the hydrophobic resin is in the range of from 0.01 to about 5 wt.-%, preferably from 0.05 to about 5 wt.-%, more preferably from 0.1 to about 2 wt.-%, , each based on the final product.

6. The composition according to any of the preceding claims, wherein the amount of the blocking agent is in the range of from 0.005 to 1 wt.-%, preferably 0.01 to 1 wt.-%, more preferably 0.01 to 0.6 wt.-%, more preferably 0.1 to 0.5 wt.-%, each based on the final product.

7. The composition according to any of the preceding claims, which is an aqueous formulation having a water activity value between 0.2-1, preferably between 0.7-0.9 and more preferably of about 0.8.

8. The composition according to any of the preceding claims, which is a food product, a pharmaceutical product, a cosmetic or an oral composition.

9. The composition according to claim 4 or 7, which is a product for oral hygiene, a tooth paste, a gel, a tooth powder, a mouth wash, a chewing gum, a tablet or a lozenge.

10. A method of producing a composition according to any of the claims 8 or 9, which comprises:
preparing a composition comprising cGMP,
adding an agent chemically blocking functional groups in cGMP and a hydrophobic resin, and/or
adjusting the pH to a value in the range of from about 3 to about 7.

11. Use of a composition according to any of the claims 1 to 7 in the manufacture of a medicament or a composition for treating or preventing caries, plaque formation, dental diseases, diseases of the mouth cavity or gums.

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K7/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A23C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 853 704 A (GAFFAR ABDUL ET AL) 29 December 1998 (1998-12-29) column 2, line 25 - line 67 column 3, line 6 - line 22 column 3, line 48 -column 4, line 8 column 4, line 43 - line 67	1,4,7-11
X	EP 0 575 121 A (ROHM & HAAS) 22 December 1993 (1993-12-22) page 2, line 38 - line 58 page 3, line 13 - line 18 page 3, line 32 - line 49 page 4, line 28 - line 32	1,2,5-11

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4 975 289 A (AMEYAMA MINORU ET AL) 4 December 1990 (1990-12-04) column 2, line 49 - line 68 column 5, line 8 - line 24 column 6, line 41 - line 50	1,4,8-10

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Information on patent family members

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